

AD-A266 093



PORT DOCUMENTATION PAGE

2a. SECURITY CLASSIFICATION UNCLASSIFIED		1d. RESTRICTIVE MARKINGS	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE UNCLASSIFIED		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.	
4. PERFORMING ORGANIZATION REPORT NUMBER USAF/DA-2		5. MONITORING ORGANIZATION REPORT NUMBER(S) AFOSR 18	
6a. NAME OF PERFORMING ORGANIZATION Dept of Physical Sciences Southeastern Okla State Univ		7a. NAME OF MONITORING ORGANIZATION AFOSR/NL	
6b. ADDRESS (City, State and ZIP Code) Department of Physical Sciences SEOSU, Station A Durant, OK 74701-0609		7b. ADDRESS (City, State and ZIP Code) 110 Duncan Ave Suite B115 Bolling AFB DC 20332-0001	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION AFOSR		8b. OFFICE SYMBOL (If applicable) NL	
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER F49620-92-J-0191		10. SOURCE OF FUNDING NOS	
11. TITLE (Include Security Classification) (See continuation sheet attached)		10. SOURCE OF FUNDING NOS	
12. PERSONAL AUTHOR(S) John R. Wright, Ph.D.		10. SOURCE OF FUNDING NOS	
13a. TYPE OF REPORT Final		13b. TIME COVERED FROM 1 Apr '92 to 31 Mar '93	
14. DATE OF REPORT (Yr., Mo., Day) May 24, 1993		15. PAGE COUNT 52 total	
16. SUPPLEMENTARY NOTATION 93 6 0 8			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary)	
FIELD GROUP SUB. GR.		18. SUBJECT TERMS (Continue on reverse if necessary)	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) A melanin-like polymer was prepared by diazotizing luminol (5-amino-2,3-dihydro-1, 4-phthalazinedione) and 3-amino-L-tyrosine in a mixed water/dimethylsulfoxide solvent; this was followed by acetone precipitation and aging of the solid residue for a month (the polymer forms slowly in the solid residue). An aqueous solution containing 0.50 F NaOH, 0.0011 M luminol, 0.010 F potassium formate and the polymer at a concentration of 0.050 mg/mL was observed to undergo an instantaneous seven-fold increase of chemiluminescence when the solution was subjected to a 0.5 Watt/mL irradiation with 20 kHz acoustic energy. This sonochemiluminescent property suggests that earlier reports of strobes of luminescence induced by pulsed microwave irradiations of aqueous solutions of the polymer might have an origin in the microwave acoustic effect. Since the sonochemiluminescent effect is marked and easily detected, the polymer may be applicable as a dosimeter for studying acoustic effects in pulsed, high-powered microwave irradiations, especially where the target geometry is complex. Continued on attached page...			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT. <input type="checkbox"/> OTIC USERS <input type="checkbox"/>		21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL Dr. Walter Kozumbo		22b. TELEPHONE NUMBER (Include Area Code) (202) 767-5021	
22c. OFFICE SYMBOL NL			

Continuation sheet

11. [Title]

"NMR Characterization of Polymers Formed in Diazotizing Mixtures of Luminol and 3-Amino-L-tyrosine"

19. [Abstract continued]

Further insight into the observed properties will depend on a characterization of the structure of the polymer, which is currently not known. Attempts at a structural characterization have been hampered thus far by a lack of suitable polymer fragments, i.e., a reliable means for fragmenting the polymer or limiting its DP_n has not been found.

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NMR Characterization of Polymers Formed in Diazotizing
Mixtures of Luminol and 3-Amino-L-tyrosine

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FINAL REPORT

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Date Submitted:	May 25, 1993
Report Due Date:	May 31, 1993
Contract No:	F49620-92-J-0191

ACKNOWLEDGEMENTS

The students and the P.I. involved in this research project wish to express their gratitude to the United States Air Force and the Air Force Office of Scientific Research for funding support and an opportunity to explore an interesting area of chemistry. Interaction between the P.I. and Dr. Johnathan Kiel at the Armstrong Lab, Brooks AFB, was essential, and assistance on the part of Lt.Col. Jan Cervený, Dr. Walter Kozumbo and the staff at Bolling AFB was greatly appreciated.

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1. INTRODUCTION

In earlier studies it was found that diazotization of mixtures of luminol and 3-amino-L-tyrosine (3AT) using acidic NaNO_2 in a dimethylsulfoxide/water solvent system produced, initially, a yellow-brown solution containing diazonium derivatives of the two aromatic amines (1). Acetone precipitation of these products was followed by a slow reaction (over a period of weeks) which lead to a brown, anionic polymer (2). The latter substance was given the name diazoluminomelanin (DALM) on the basis of its color and its origin from a substituted tyrosine (3). The resemblance to a melanin could be superficial since DALM has not been established as a melanin on the basis of molecular structure. A similar polymerization was observed for the diazotization product of 3AT alone (hereafter DAT for diazotized 3AT), which also produced a brown, anionic polymer under a variety of reaction conditions, including a slow reaction in the original diazotizing solution. Both polymers, DALM and DAT, exhibited typically broadened nmr spectra.

Aqueous, alkaline solutions of DALM were chemiluminescent over a period of hours if the solution contained about 1% by weight H_2O_2 . This chemiluminescence was temperature dependent (also sensitive to dissolved CO_2 or carbonate anion) and could be the basis of a thermal imaging dosimeter for microwave applications. The behavior of aqueous, alkaline DALM containing both H_2O_2 and the carbonate anion was even more interesting since pulsed microwave fluences produced a bright strobe of chemiluminescence (4) under conditions

which did not cause significant heating on the sample. This transient phenomenon may have derived from the so-called microwave acoustic effect (5), i.e., the deposition of a narrow pulse of microwave energy has been shown to induce an acoustic wave, and this could have lead to sonochemiluminescence. Various investigators have thought that sonic waves, if they are intense enough, might induce homolytic bond cleavage through large, transient deviations from the apparent temperature, a result of cavitations in the target material (6), thus producing free radicals which could activate any chemiluminescent species present (such as luminol or its derivatives). An alternative explanation held that sparks in the waveguide might have produced a pulse of ultraviolet radiation, in turn inducing fluorescence in the sample. However, control experiments involving barrier filters and fluorescent substances appeared to eliminate the possibility (4). Also, the observed effect depended on the presence of DALM in the target solution, and the latter solutions emanated an audible "pop" when the pulse of microwave energy was applied.

2. OBJECTIVES

The two priority objectives of the project have been 1.) further structural characterization of the polymer formed in diazotized mixtures of 3-AT and luminol (DALM); included in this objective was a parallel characterization of the polymer of diazotized 3-AT alone, which was thought to possess a polymeric structure similar to that of DALM and 2.) a characterization of the

chemiluminescent properties of DALM, especially with regard to a possible sonochemiluminescent response. An additional objective focused on chemiluminescence enhancing effects of metal cations upon solutions of DALM. These were the main objectives agreed upon with Dr. Johnathan Kiel during a 1991 coordination visit at the sponsoring laboratory at Brooks AFB, Texas.

3. EXPERIMENTAL METHODS

3.1. Materials

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), sodium nitrite and 3-amino-L-tyrosine were obtained from Sigma Chemicals, and potassium formate was purchased from Aldrich Chemicals (stock number 29,445-4). Other substances used in the investigation were obtained from Sigma, Aldrich or Fluka.

3.2. Preparation of the DALM Polymer

The synthesis protocol for DALM, as it was used here, is presented in Figure 1. In previous work (2) it was shown by means of differential scanning calorimetry that the exotherm due to unreacted diazonium groups fades away over a period of weeks when the DALM-forming mixture is aged at room temperature, and this decomposition reaction corresponded to the onset of polymer formation. Decomposition of the diazonium species may also be followed by means of nmr spectroscopy.

DALM PROTOCOL

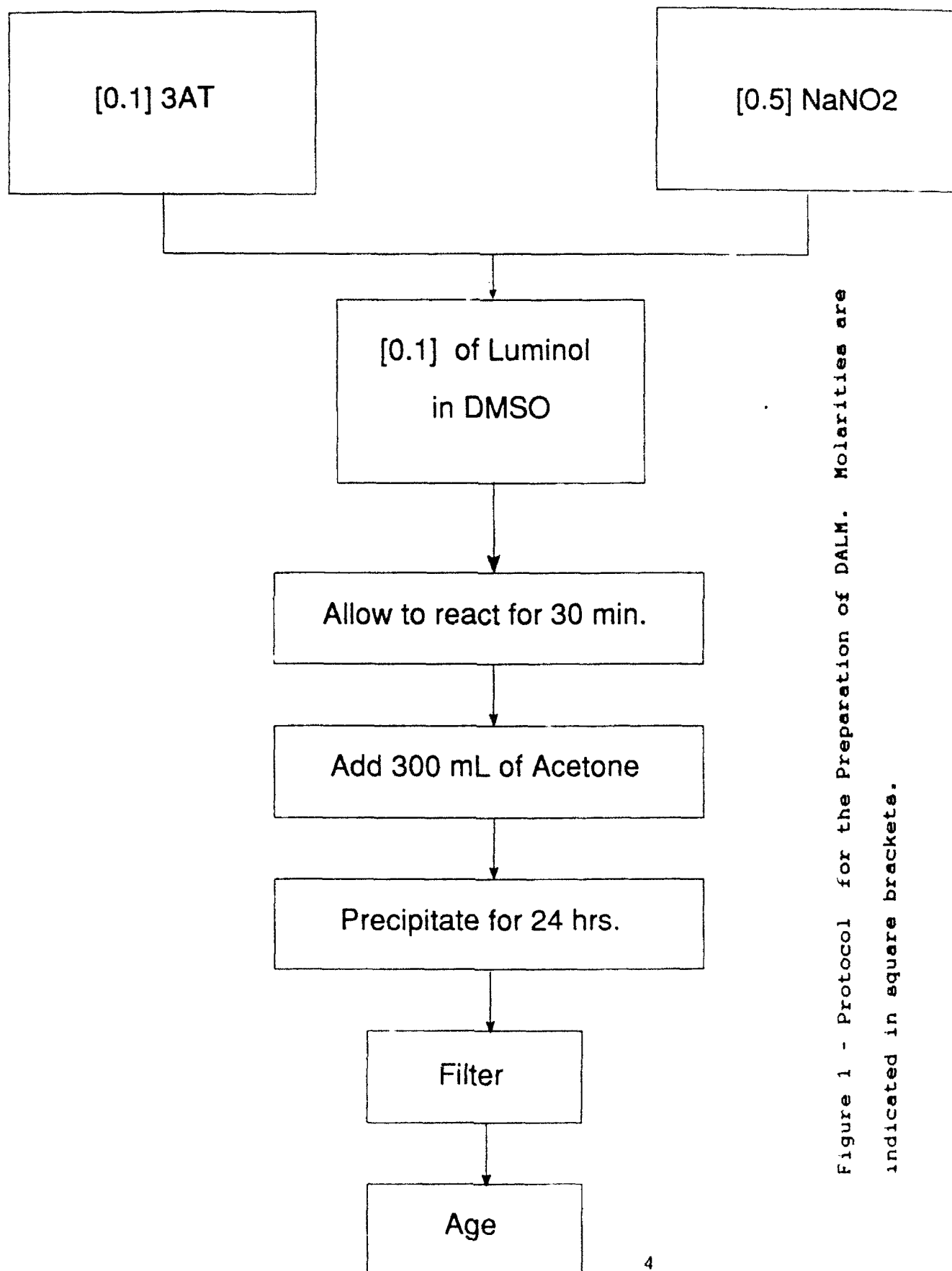


Figure 1 - Protocol for the Preparation of DALM. Molarities are indicated in square brackets.

During the current reporting period it was determined that the chemically-induced chemiluminescence properties of the DALM-forming mixture varied very little over the initial 45 day period. 5.0 mg samples were removed periodically from three separate preparations of DALM and subjected to a standard chemiluminescent assay. A linear least squares analysis of the data yielded the following empirical equation:

$$I = -0.64 t + 192$$

where I is the luminometric intensity (instrument scale) and t is the aging time in days. Standard deviations of the slope and intercept were, respectively, 1.32 and 31. The slope could have a low positive or negative value, depending on the choice of including all data or eliminating the more deviant measurements.

The DALM polymer used in the sonochemiluminescence experiments reported here was selected from preparations which had aged at least three weeks.

3.3. Preparation of the Polymer of 3AT (DAT)

This polymer (DAT) was prepared by diazotizing 3AT in a pure water solvent. A mixed solvent containing DMSO was not necessary since 3AT is soluble in water. Nmr spectra confirmed that the immediate reaction product was the diazonium derivative of 3AT, and the nmr resonances of the latter substance gave way to the broadened resonances of the polymer over a period of several days.

After a ten day reaction time the polymer was precipitated using excess acetone.

A very similar (if not identical) polymer was obtained by the method of Figure 1, with DMSO omitted.

3.4. Measurements of Sonochemiluminescent Effects

Figure 2 shows a salient drawing of the locally constructed cabinet in which sonochemiluminescent measurements were performed. In the experiments with metal oxide dosimeters a relay lens was used to focus light from a very specific region, e.g., just below the transducer, upon the end of the fiber optic bundle. This was done for the metal systems because sonochemiluminescence occurred almost entirely in the high amplitude region near the transducer's tip. In the experiments with various anions and DALM, the emanation of sono-chemiluminescence was more diffuse, though still brighter close to the transducer's tip. In these cases the relay lens was removed, and the fiber optic bundle was pulled through the aperture and placed in contact with the sample cuvette.

Figure 3 shows the fiber optic adapter which was used to couple light coming from the bundle into the Turner TD-20e's photodetector. The chrome plated sphere (diameter 2.0 mm) was located at the focus of the cell's paraboloid reflector; this arrangement couples more than 70 percent of the exiting light into the photomultiplier.

In order to avoid violent agitation in the sample solutions,

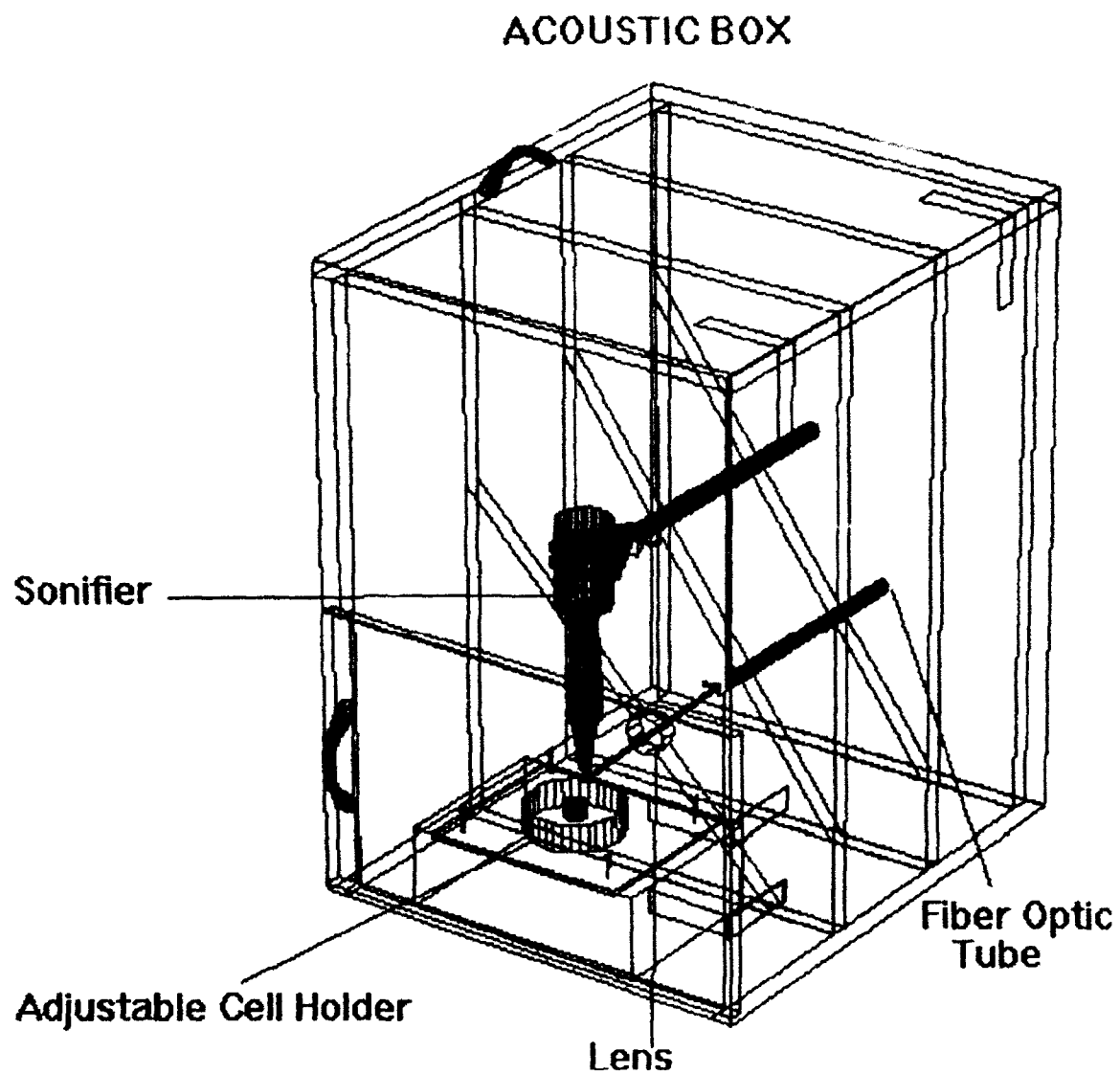


Figure 2 - Sonic Irradiation Cabinet (constructed locally)

LUMINOMETER CELL ADAPTOR

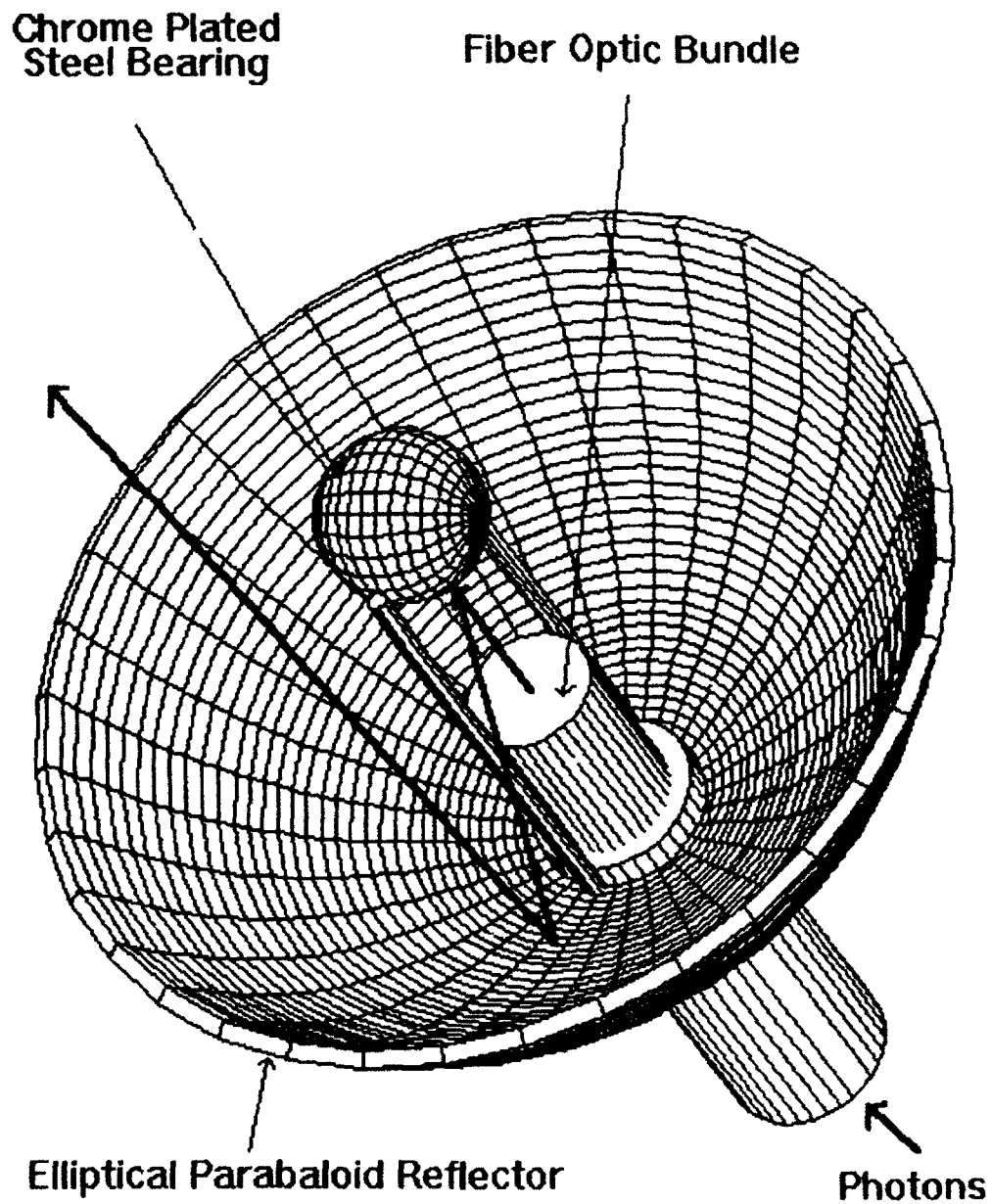


Figure 3 - Turner Luminometer Fiber Optic Adapter (constructed locally)

sonic irradiations were held to 50 Watts (at 20 kHz) from the Branson W-185 sonifier system.

3.5. Dosimeter Preparations and Luminometric Procedures

3.5.1. Hydrated Metal Oxide Systems

Transition metal dosimeters were prepared by dissolving 20.0 mg of luminol in 6 mL of 1.0 M NaOH (aqueous). A 3.00 mL aliquot of this stock solution was then added to 47.0 mL of deionized water and mixed thoroughly; this was solution A. Solution B was prepared by dissolving 250 microLiters of a 2 percent (weight/volume) solution of the transition metal salt in 50 mL of aqueous 3 percent H_2O_2 . Solutions A and B were then mixed. After an arbitrary one minute forty five second delay from the mixing time, a quiescent luminometric reading was obtained (I_0). Then the acoustic irradiation was started, simultaneously with a second luminometric measurement (I). Sonochemiluminescent effects were recorded as the ratio I/I_0 .

3.5.2. Systems Based on Anions and the DALM Polymer

Measurements of sonochemiluminescent effects of various anions and DALM in luminol-containing solutions began with 100 mL aliquots of aqueous 0.50 M NaOH containing 20.0 mg of luminol; these also contained weighed amounts of sodium or potassium salts of the

particular anion in question (i.e., to obtain a desired anion molarity when the solutions were made up to their final 100 mL volume).

The aliquot was placed in the irradiation cell, and with the cabinet closed to external light, luminometric measurements were recorded at a repetition rate of one per 15 seconds. Six readings were obtained with the sonic power off, followed by an additional 14 with the power on. The power was then turned off and a 0.50 mL aliquot of three percent H_2O_2 was stirred into the cell. The cycle of six measurements off and 14 on was repeated, and the power was again turned off. A weighed quantity of 5.0 mg of DALM was then stirred into the cell, and a final set of six measurements were recorded with the sonic power off followed by 14 more with the power on. All experiments were replicated for a minimum of four times.

3.6. Nmr Measurements

Nmr spectra were obtained by means of a 4.7 Tesla Chemagnetics A200 liquids system which has been modified for 2D applications. The available 2D data acquisition pulse and phasing programs include H,H-COSY, H,C-COSY (HETCOR), J-resolved hydrogen spectroscopy, MKE spectroscopy and 2D-INADEQUATE (double quantum) spectroscopy. Other versions of 2D nmr are easily added since the instrument's operating system includes developmental software. The instrument is broadband and tunes the range from nitrogen to phosphorous.

3.7. Computations and Data Analysis

Two dimensional nmr spectra were processed using FELIX software and an ALR 80386/387 system with a 120 MB hard disk and a 16 MB RAM drive. Various computations and luminometric data presentations were accomplished with a similar office machine, using a variety of licensed programs (e.g., SlideWrite, Mathcad, Huckel MO Calculator, several statistical analysis programs, etc.).

4. RESULTS AND DISCUSSION

4.1. Sonochemiluminescence and DALM

4.1.1. Metal Cations and DALM

Alkaline aqueous solutions containing luminol and transition metal ions in the form of hydrated oxide colloids and prepared as described in 3.5.1. exhibited varying degrees of sensitivity to acoustic irradiations measured as a ratio of luminescence after applying the sonic field to that immediately before the irradiation, I/I_0 . The metals tested in these experiments include those of Table II (see 4.2.) and, in addition, zirconium(IV) and iron(III). In all cases the addition of 5.0 mg of DALM to the 100 mL quantity of solution prior to irradiation quenched sonochemiluminescence.

Part of the observed quenching effect is due to the pigment absorption of DALM, which is considerable even at 5 mg/100 mL,

e.g., the absorbance at 450 nm was measured at 0.77, and the chemiluminescence emission is blue. Also, the phenolic groups of the 3AT structure, granting that they are still present in the polymer, should behave as free radical scavengers and thus interfere with the initiation of luminol's chemiluminescence (7). In view of these properties the chemiluminescent behavior of the DALM polymer would seem to be something of a surprise. There is no question that phenolic materials are able to scavenge initiating radicals; e.g., the sonochemiluminescent effects of the above-described metal ion systems were thoroughly quenched by added 3AT, which does not absorb visible light, and 3AT also quenched the anion chemiluminescent effects to be described in the following part.

4.1.2. Anions and DALM

The effect of anions on luminol sonochemiluminescence has been known at length (8). For example, alkaline solutions of luminol containing the carbonate anion exhibit a pronounced sonochemiluminescence compared with solutions of luminol in aqueous NaOH. The possibility that anions might interact with DALM was given some thought, and several anions were examined in solutions containing alkaline luminol and H_2O_2 , both with and without added DALM, using the procedures of 3.5.2. The selected anions were carbonate, formate, phosphate, hypophosphite, nitrate, nitrite, sulfate and chlorite. The most pronounced sonochemiluminescent effects for alkaline luminol in the absence of DALM or H_2O_2 were

observed in the presence of phosphate, carbonate or formate anions, whereas the other anions taken into consideration exhibited a more attenuated sonochemiluminescence. As shown in Table I, these are very large ratios of I/I_0 , and the ratios are seen to be sensitive to the anion's concentration. Optimum concentrations exist for all three anions. The "water blank" (luminol in aqueous 0.5 M NaOH or KOH) is also sensitive to acoustic irradiation, but a dissolved CO_2 effect is probably acting since the apparatus for conducting sonic irradiations is not designed to exclude atmospheric gases for any length of time.

TABLE I - Sensitization of the Sonochemiluminescence of
Luminol Solutions by Simple Anions

<u>Mean Values of I/I_0 at 50 Watts*</u>				
<u>Anion</u>	<u>1.0 M</u>	<u>0.10 M</u>	<u>0.010 M</u>	<u>0.0010 M</u>
phosphate (insol.)		3613	951	-
carbonate	294	786	38	-
formate	36	49	583	87

* I (irradiation ON); I_0 (irradiation OFF). The water blank control with alkaline luminol and either K^+ or Na^+ as counterion to the anionic luminol species averaged an I/I_0 value of 286. The relative standard deviation of these data was about 18%.

As described in 3.5.2., evaluations of the effect of DALM in the presence of the above described anions were conducted in a sequential manner. The intermediate step of adding H_2O_2 before DALM was included since the experiments at Brooks AFB (which revealed

the strobe of luminescence during pulsed microwave irradiations) were carried out with a small amount of H_2O_2 added to the DALM/carbonate solution (4). The addition of H_2O_2 caused a further increase in the observed chemiluminescence of luminol and a less pronounced relative increase on irradiating with sonic energy. The polymer apparently quenched acoustic effects in solutions of luminol and H_2O_2 containing either carbonate (Figure 4) or phosphate (not shown but essentially the same as Figure 4), but in the case of formate, a distinct, transient burst of light was seen on applying acoustic energy (Figures 5 - 7). The effect with formate is intrinsically brighter than these figures indicate since the absorbance due to the polymer is substantial ($A_{450} = 0.77$). The formate effect was largest at 0.01 M (compare Figures 5 - 7). A control sample with nitrite in place of DALM did not produce a sonochemiluminescent step (Figure 8); an effect due to nitrite had to be considered since sodium nitrite is a possible contaminant in DALM preparations. The control experiment of Figure 9 resembles that of Figure 4. These are reproducible results, as revealed by replications of all of the experiments. The data thus appear to implicate just the formate anion and the polymer as the sources of the sonochemiluminescent effect exemplified by Figures 5 - 7.

In the 0.01 M formate system containing luminol, H_2O_2 and DALM the relative increase (quiescent to irradiated) is approximately seven-fold, but the peak intensity of the latter system is approximately thirty six-fold greater than that of the simple system of luminol and formate with DALM and H_2O_2 missing. The greater luminescence is evident when the irradiation is observed

Figure 4 - Sonochemiluminescent Effects with Carbonate ion at 0.01 M (data at other concentrations were similar)

Potassium Carbonate (0.01 M) with Luminol, H₂O₂, and DALM added

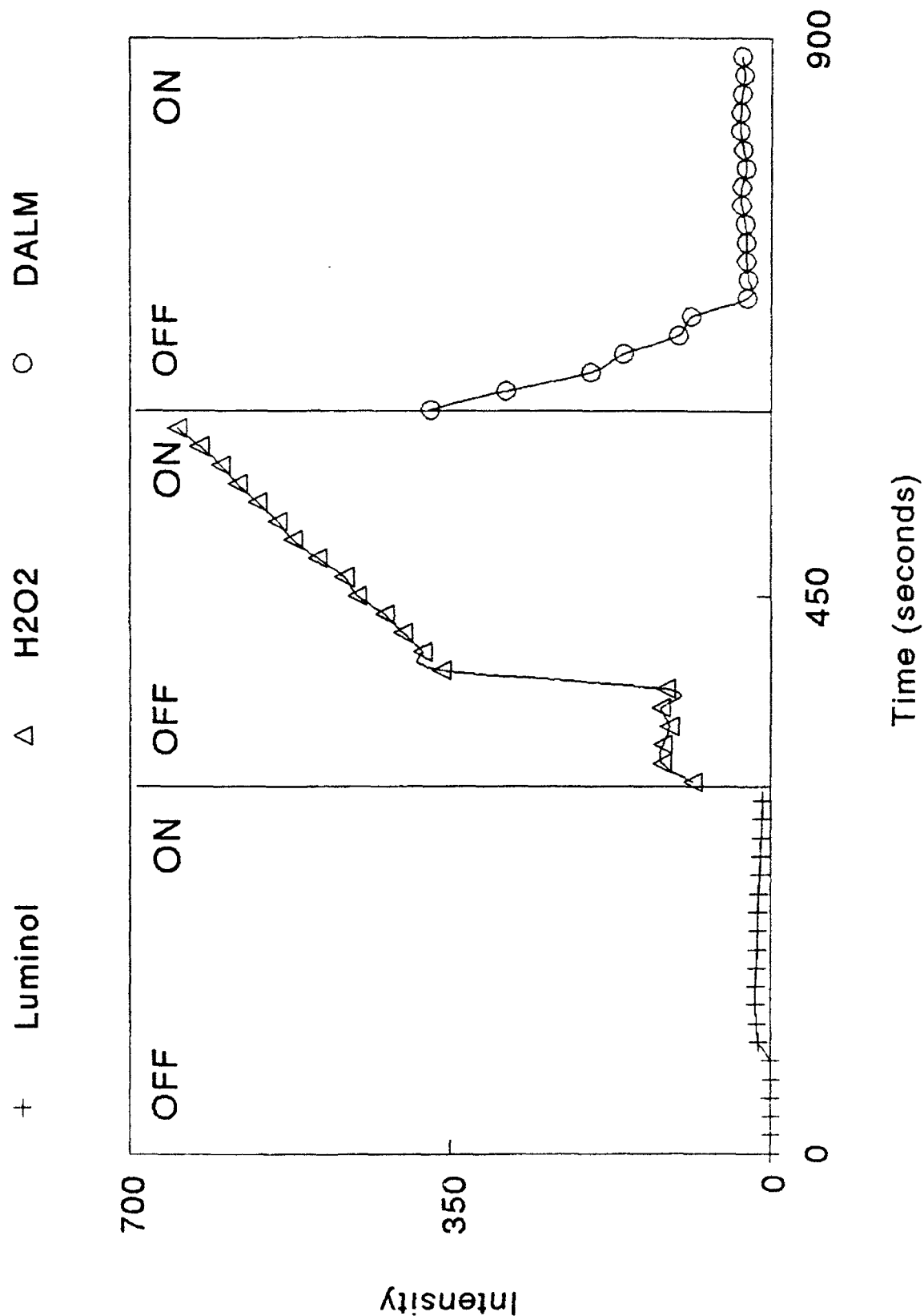
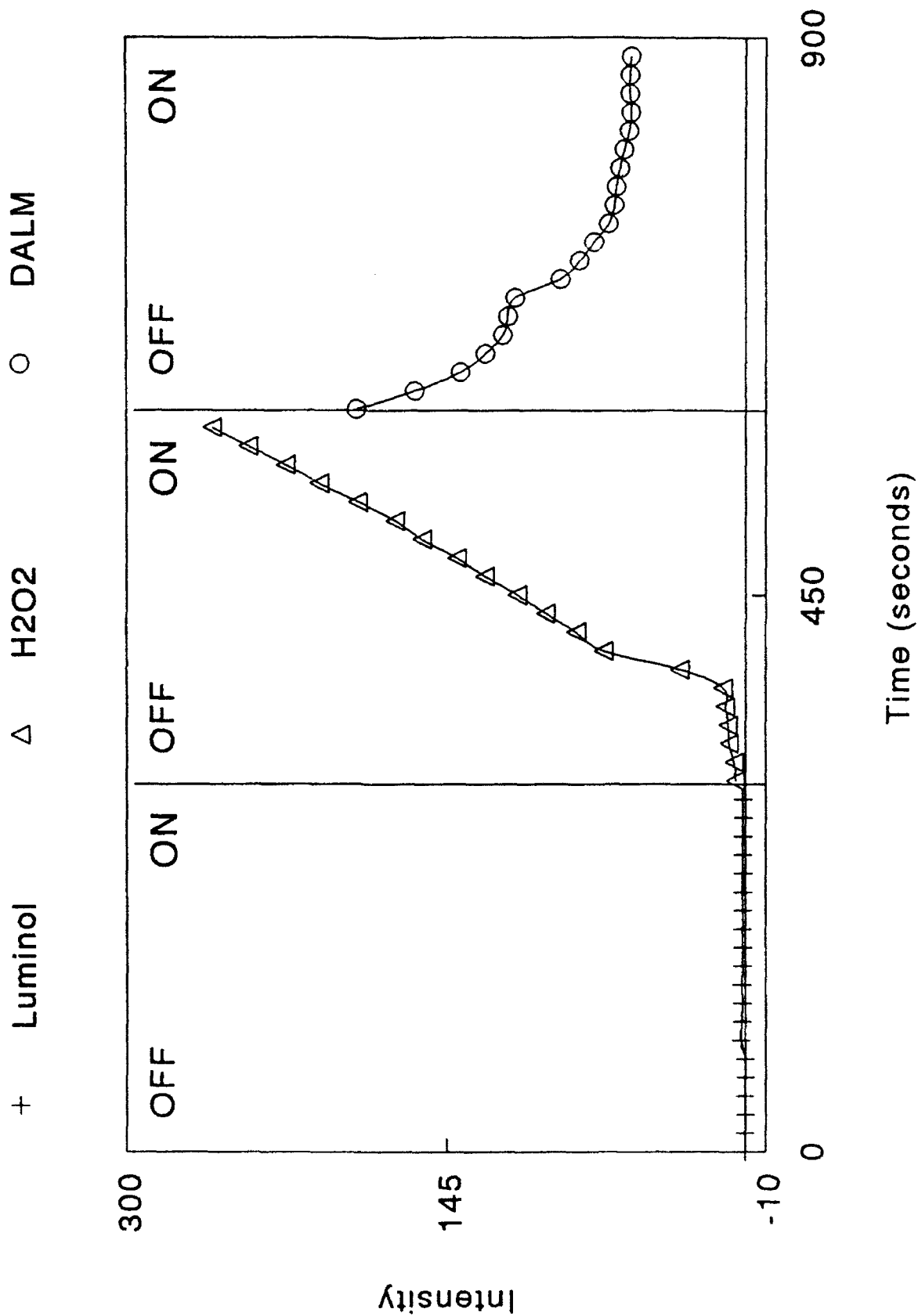


Figure 5 - Sonochemiluminescent Effects with Formate Ion at
1.0 M

Formate (1 M) with Luminol, H₂O₂, and DALM added



Formate (0.1 M) with Luminol, H2O2, and DALM added

Figure 6 - Sonochemiluminescent Effects with Formate Ion at
0.10 M

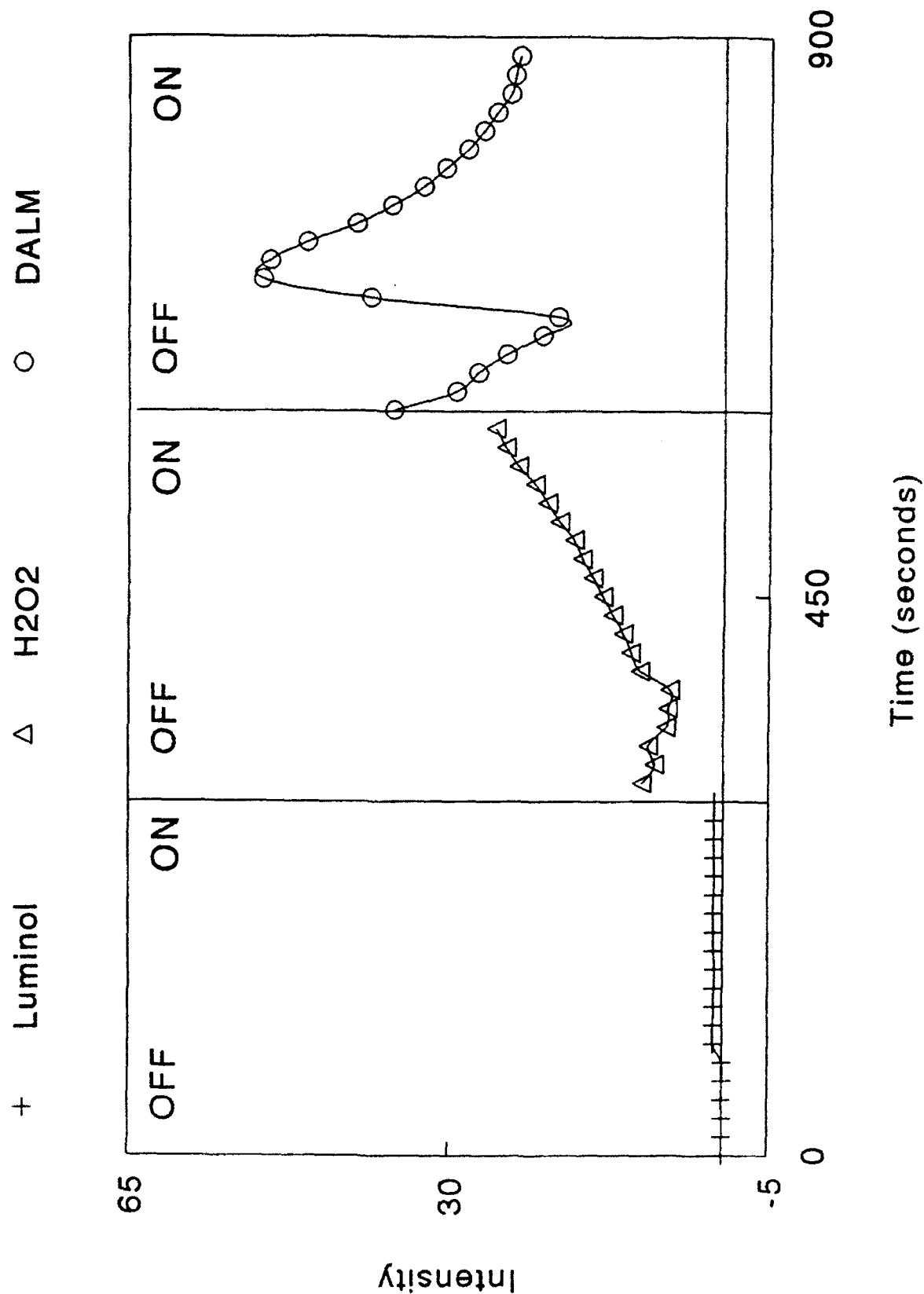


Figure 7 - Sonochemiluminescent Effects with Formate Ion at
0.010 M

Formate (0.01 M) with Luminol, H2O2, and DALM added

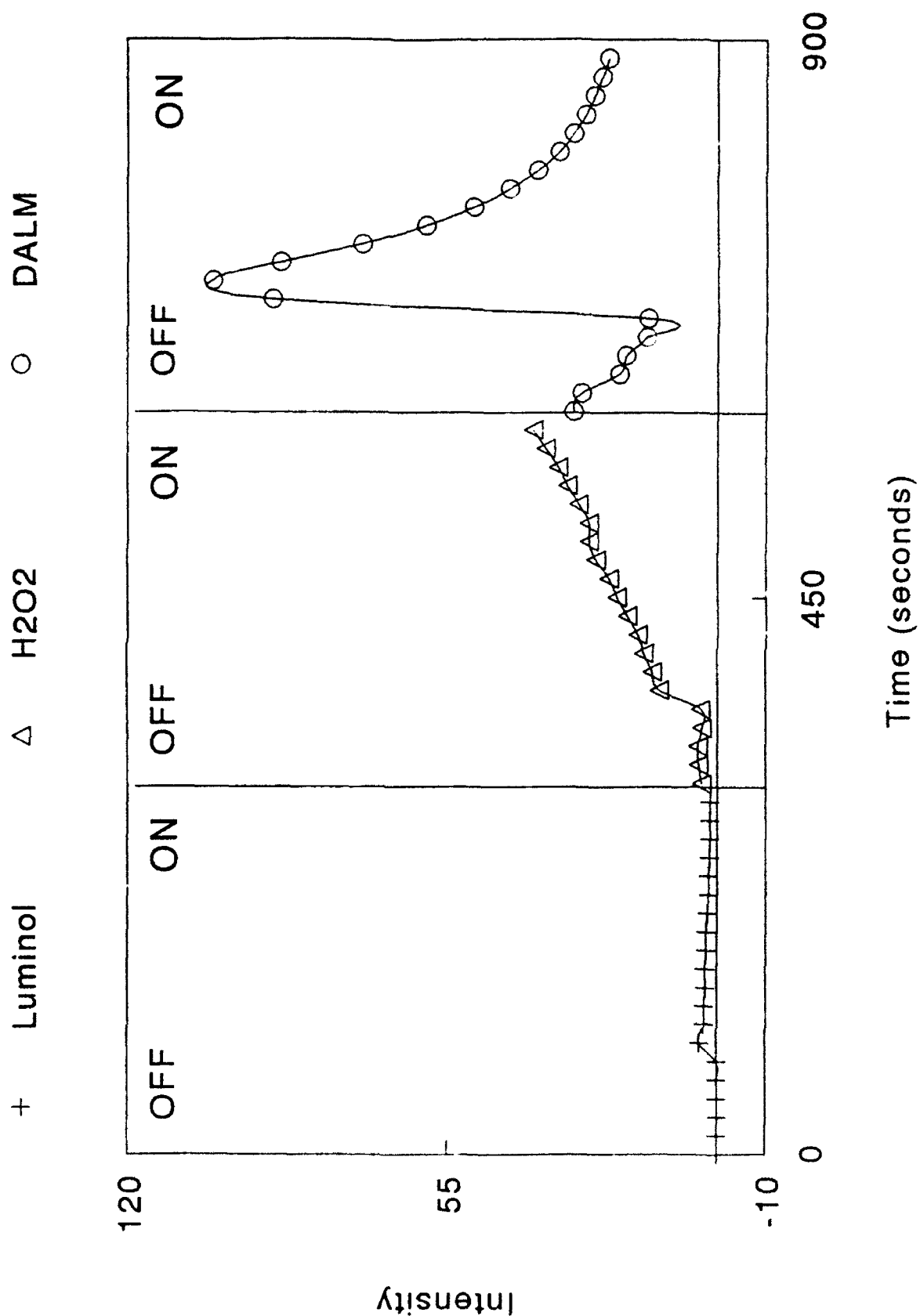
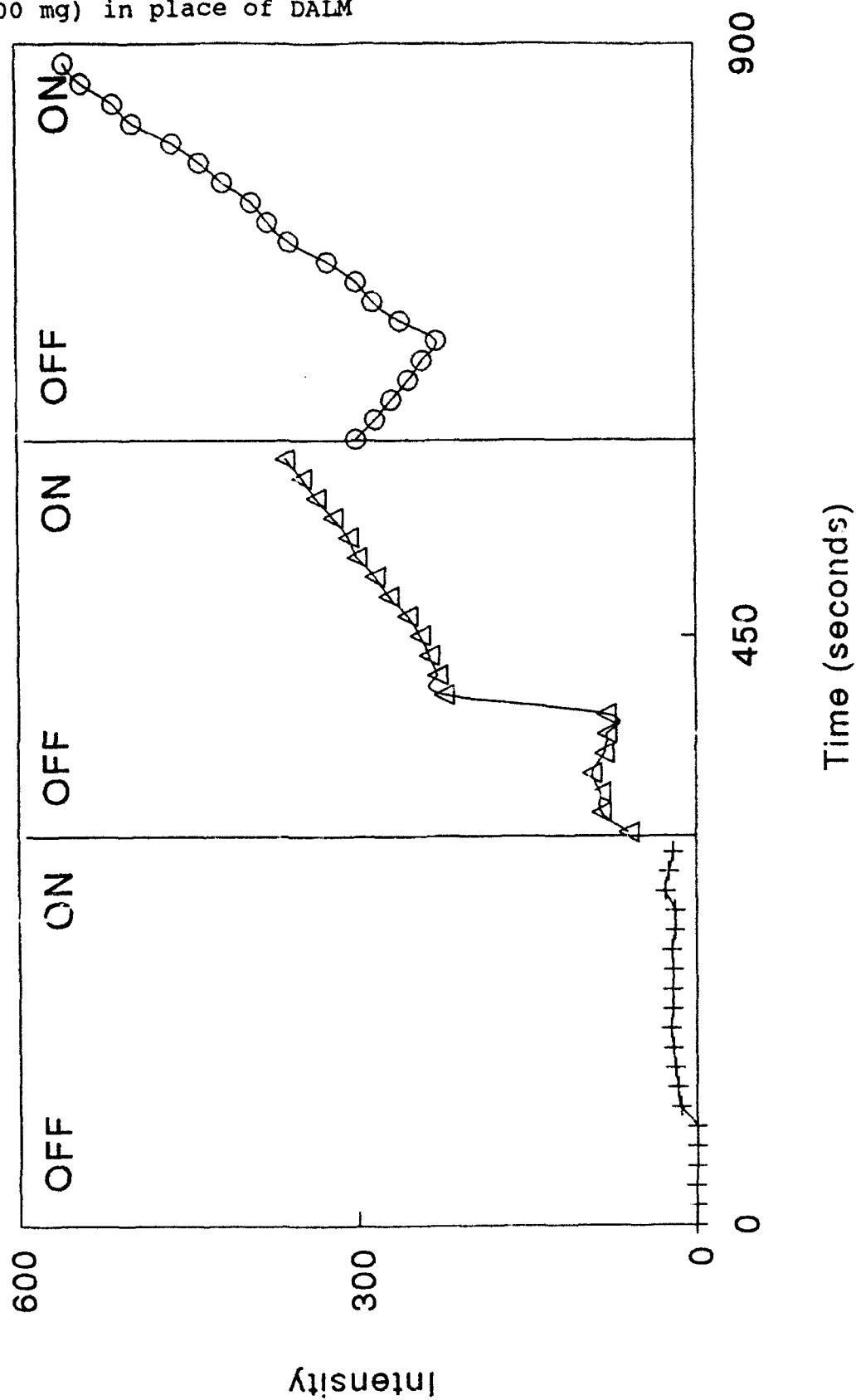


Figure 8 - Sonochemiluminescent Effects with Sodium Nitrite

(5.00 mg) in place of DALM

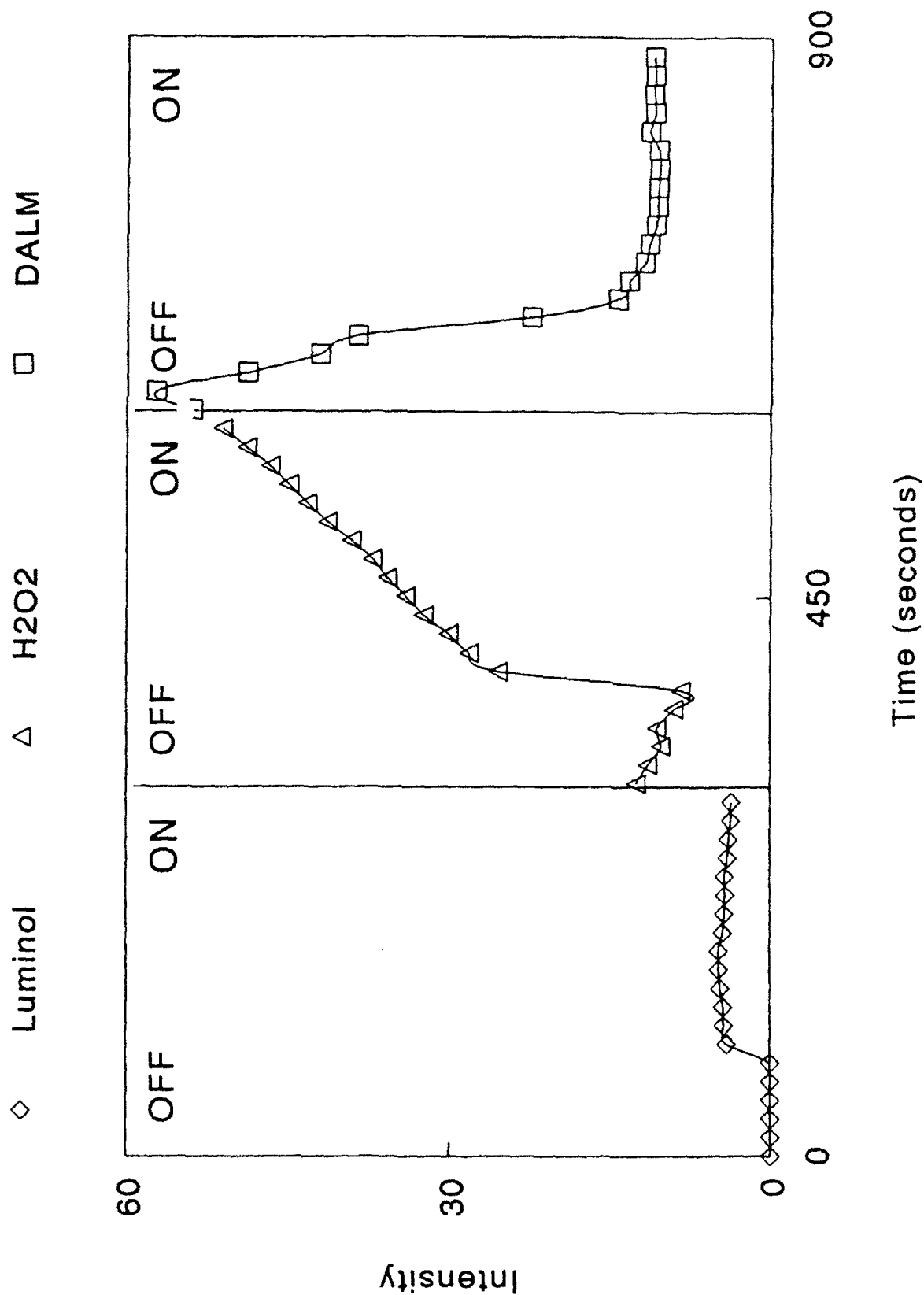
Formate (0.01 M) with Luminol, H₂O₂, and NaNO₂ added

+ Luminol Δ H₂O₂ ○ NaNO₂



WATER BLANK with Luminol, H2O2, and DALM added

Figure 9 - Sonochemiluminescent Blank Experiment



visually. It is not clear why formate in the presence of DALM should enhance sonochemiluminescence, but possible explanations might include the charge (or absence of charge) on initiating radical species coming from or interacting with the DALM polymer. The evidence does clearly establish that DALM markedly enhances sonochemiluminescence under certain conditions, leading one to suspect that the transient luminescent effects observed in pulsed microwave irradiations of DALM solutions (4) have an acoustic origin.

The formate/DALM sonochemiluminescent effect was very nearly missed since the first experiments made use of an older sample of potassium formate which had been in stock for about a decade and which caused no sonochemiluminescent effects (the sample had been accepted in lieu of waiting weeks for an order to arrive). The technician then noticed the receipt date marked on the container and requested a fresh sample of potassium formate; then a repeat of the former experiments revealed the sonochemiluminescent effects reported here. The difference appears to be the result of an impurity which accumulates on aging, which must be taken into account in any future studies of this system. All of the data presented in this report were obtained from fresh materials.

4.2. Simple Sonochemiluminescent Metal Ion Dosimeters

A small effort was directed to further characterizing simple metal oxide dosimeters (i.e., without added DALM) which respond to acoustic irradiation, and since the work was conducted by a new

student, some of the dosimeters previously characterized were included with the new systems for control purposes. The results are presented in Table II, and the instantaneous enhancements of chemiluminescence (I/I_0) on applying 20 kHz acoustic energy at 50 Watts was closely comparable to published values (9). The earlier mean value for cupric chloride ($I/I_0=1.1$ s.d. 0.04) was less than the result for cupric sulfate ($I/I_0=1.4$ s.d. 0.41) but within the experimental error of the latter measurements. The increase may also be real, resulting from the difference of an oxygenated anion (10) since the correlation of fairly large sonochemiluminescent enhancements with anion concentration has been a consistent result (see 4.1.2.).

Nickel chloride produced the largest response, but its reproducibility was poor for some undetermined reason. The same kind of scatter was observed in a separate set of twelve replications.

4.3. Structural Investigations: Nmr Considerations

Evidence gathered during the previous funding period (2) seemed to favor an aryl amine polymer linkage of the type shown here, both in DALM and in the DAT polymer:

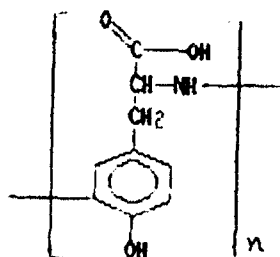


TABLE II - Sensitization of the Sonochemiluminescence of
Luminol Solutions by Metal Ions/Metal Oxides

<u>COMPOUND (Ion Source)</u>	<u>I/I₀*</u>	<u>Standard Deviation</u>
nickel(II)chloride	29.12	13.92
oxovanadyl(IV)sulfate	8.68	6.99
vanadium(III)chloride	3.00	0.56
gadolinium(III)chloride	8.32	2.02
chromium(III)chloride	6.68	0.48
cadmium(II)chloride	6.33	4.77
cadmium(II)sulfate	1.87	0.09
barium(II)phosphate**	5.07	0.91
palladium(II)chloride	2.55	0.50
scandium(III)chloride	2.19	0.13
cobalt(II)sulfate	2.09	0.83
potassium carbonate***	1.61	0.33
sodium carbonate***	1.74	0.22
copper(II)sulfate	1.40	0.41

*Measured with a 22.3 °C starting temperature.

**Insoluble to begin with. Dispersed by first heating and stirring.

***Soluble ions. Probably mostly an anion effect. All of the metal compounds in Table II are dilute in the test mixtures.

It was thought that in DALM, luminol might be attached as a pendant structure, consistent with the fact that the polymerization begins with a diazonium group at the 5-position. Modifications of the dihydrophthalazinedione structure, such as polymer bonding involving the azine nitrogens, should mitigate against chemiluminescent properties, and this seemed to rule against much modification of the basic luminol structure in view of the polymer's chemiluminescent properties. In contrast, diazotized 3AT offers several reactive groups that might participate in a polymerization and in linkages to pendant groups.

The H,H-COSY spectrum of dehydrated 3AT in d-DMSO solvent (Figure 10) permits detection of the -OH, and -NH₂ protons and shows that it should be feasible to trace the connectivity between polymeric units if the suspected aryl amine structure is correct. In Figure 10 the assignment of the 1D spectral features to the structure is shown by means of lower case letters (a, b, c etc.), and a crosspeak is found between the aryl and aryl amine hydrogens in the COSY spectrum. If the aryl amine linkage is correct, then the aryl amine hydrogen should also crosspeak with the alpha hydrogen and the latter in turn with the -CH₂- protons (for which a crosspeak is observed in Figure 10); a network of connectivities of this type could establish the nature of the polymer's linkage, but such a proof would depend on the isolation of suitably small polymer fragments, which has proven to be the crux issue.

If other connectivities occur the situation may be less fortunate. For example, if a crosspeak occurs between the -CH₂- and aryl hydrogens it is so weak that it is lost in the T₁ noise in

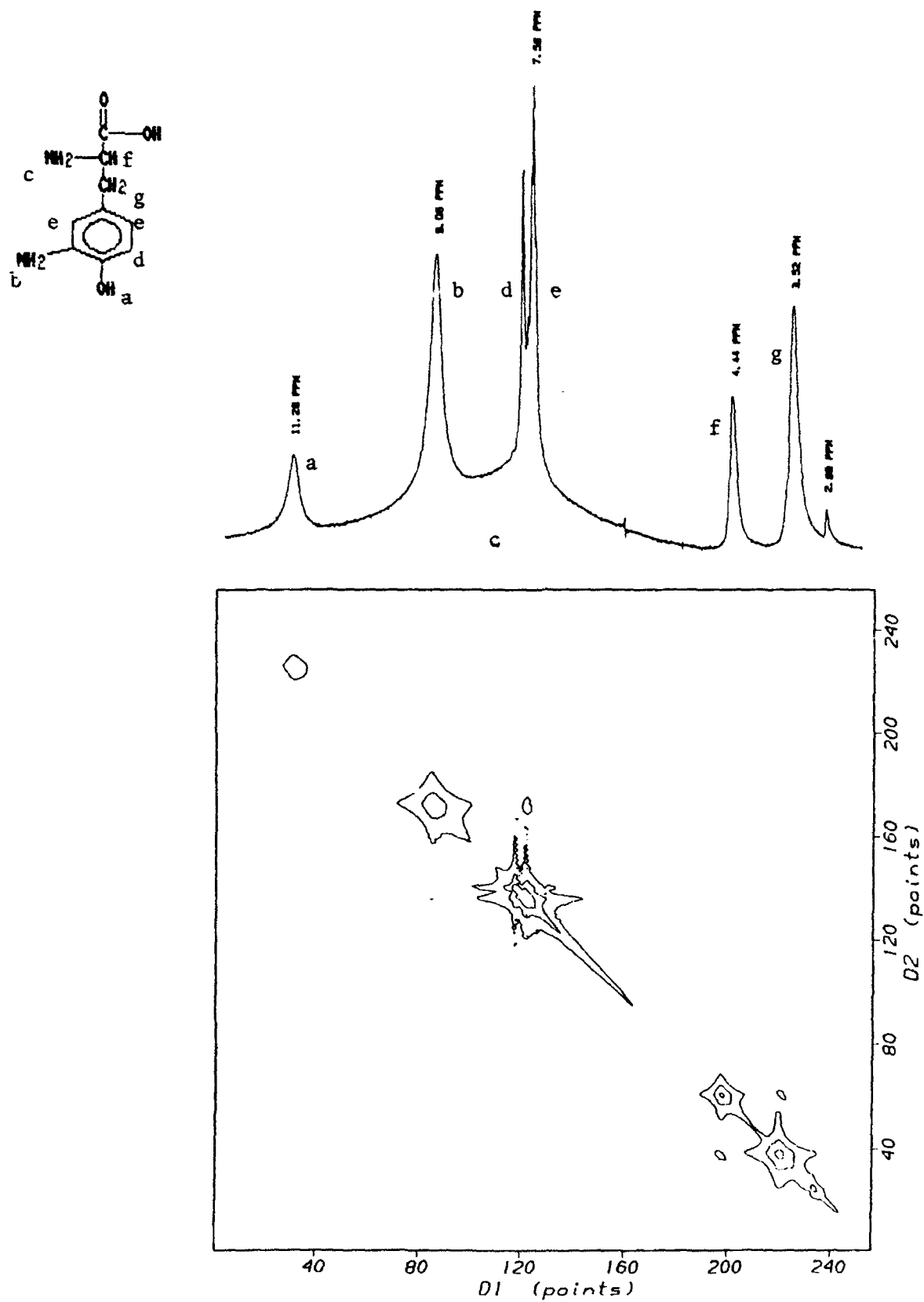


Figure 10 - 1D Hydrogen and 2D H,H-COSY Spectra of 3-Amino-L-tyrosine with Structural Assignments

Figure 10. Also, linkages placing more than three bonds between hydrogens would probably be undetectable by ordinary H,H-COSY spectroscopy.

4.4. Nmr Studies of the DALM and DAT polymers

During the current funding period attempts were made to prepare low DP_n oligomers by varying the temperature over a wide range and using brief reaction times in the DALM and DAT polymerizations (as proposed). These efforts were unproductive because in both cases the narrow resonances of the luminol and 3AT diazonium compounds gave way directly to the very broadened polymer resonances, i.e., intermediate species were never detected even when the reaction time was brief. This result is what one would expect if the polymerization proceeded by a chain addition mechanism and it is in contradiction with an aryl amine linkage (4.3.), which is expected to form in a stepwise condensation process.

Very similar results were obtained when attempts were made to truncate the polymer DP_n by including amino acids in the reaction mixture, such as glutamic acid or threonine, which could not undergo the aryl diazotization. Products obtained in this manner were rigorously dried over P_2O_5 before being dissolved in dDMSO for nmr measurements. Figure 11 presents the nmr spectrum of a DAT polymerization product which was prepared with threonine added to the reaction mixture, and it was a typical result. In spite of the drying step, the spectrum does not show the distinct -OH and -NH₂

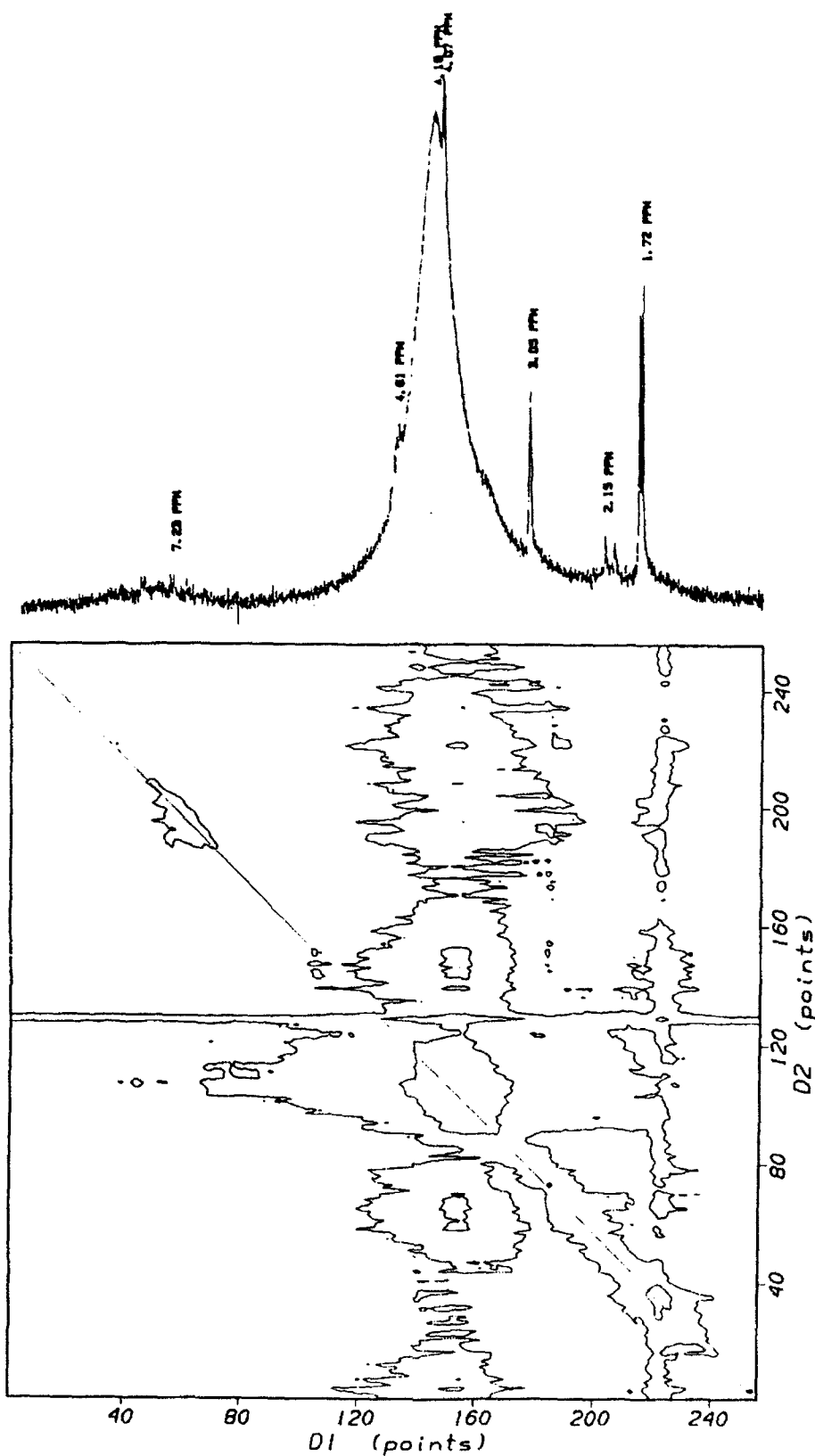


Figure 11 - Spectrum of a Polymeric Product with 3-Amino-L-tyrosine and Threonine in the Reaction Mixture
(Narrow lines upfield are unreacted threonine)

resonances observed in the case of dehydrated 3AT (Figure 10), and the aromatic resonance is broadened; the 2D nmr methods are thus uninformative. We have not exhausted all of the possibilities for truncating the polymerization, and it should be noted that the DP_n of a free radical chain polymerization may be drastically reduced in the presence of an appropriate scavenging agent (11). Scavenging methods are also applicable to ionic polymerization processes.

The funding period ran out before several untried chemical degradation methods (e.g., the $HNO_3/Ba(OBr)_2$ approach) reagent, could be tested, and chemical degradation might be the best access to this problem.

5. CONCLUSIONS AND RECOMMENDATIONS

The work reported here produced the significant finding that DALM indeed exhibits a marked sonochemiluminescent effect under appropriate conditions, and the substance could be the basis of an imaging sonic dosimeter for studying high powered microwave (HPM) acoustic effects in targets. The conditions for achieving DALM sonochemiluminescence were not identical to those used in the cited pulsed microwave study (4), which involved DALM, H_2O_2 and carbonate anion in an aqueous solution. The latter mixture did not chemiluminesce markedly in response to purely acoustic irradiations, as shown in our experiments, whereas systems containing formate in place of the carbonate anion were indeed sonochemiluminescent. However, it should be pointed out that

radicals related to the formate anion might also be produced in carbonate solutions, depending on the REDOX processes involved (10). An attempt will be made to publish these findings in a peer reviewed journal. It is recommended that the alkaline DALM/H₂O₂/formate anion dosimeter be tested in a pulsed microwave field at the Brooks AFB facility to determine if a strong transient chemiluminescence also occurs under pulsed microwave irradiations. It is further recommended that the acoustic experiments be conducted in a non-sequential manner against the possibility that sonochemiluminescent effects were missed due to depletion of the chemiluminescent agent (luminol), especially in the case of the carbonate anion; the funding period ran out before these possibilities could be tested.

All efforts to characterize the structures of the DALM and DAT polymers to date have met with frustration in spite of a considerable effort and nmr instrument use time. Progress of the structural characterization clearly depends upon obtaining low DP_n fragments of the higher molecular weight material, either by limiting the DP_n in the initial polymerization reaction or by creating manageable fragments through chemical degradation. The isolation of oligomeric species should allow nmr characterization of the linkage, especially by H,H-COSY and other 2D nmr methods.

It is recommended that future studies be focused almost entirely on a structural characterization of the DALM and DAT polymers since a better understanding of their interesting properties will depend upon structural insight. A proportionately smaller effort should be devoted to controlled experiments which

better define the sonochemiluminescent properties of DALM.

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(10) Both charge and spin delocalization may act to stabilize radical species derived from various oxygen-containing anions; see A. M. Michelson, J. Maral and C. Mony, "Carbonate Ion Effects on the Oxidation of Luminol, Oxidative Hemolysis, Gamma Irradiation and the Reaction of Activated Oxygen Species with Enzymes Containing Various Active Centers", Biochimie, 65, p95 (1983).

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7. APPENDIX I - DEVELOPMENT OF STUDENTS

The guide literature does not require this section, but it was included since research findings were not the only direct product of the project. In particular, the project's contribution to human resources were significant and should be taken into account.

The technician, Phyllis Berryman (B.S. in chemistry), has applied for admission to a graduate program in environmental health and safety at Oklahoma State University. Staci Medders, one of the participating students, received her B.S. degree in chemistry at the spring 1993 commencement, and she expects to enter the medicinal chemistry doctoral program at the University of Oklahoma this fall. The other student, Danny Rose, has been admitted to the graduate program in biochemistry at Rice University. Danny also received his B.S. degree in chemistry, with honors, during the spring 1993 commencement. The abstracts of three papers which were presented by these individuals at a local American Chemical Society meeting (April 1993) are included with this appendix.

DIAZOTIZATION POLYMERIZATION OF 3-AMINO-L-TYROSINE AND LUMINOL, Phyllis Berryman, Danny Rose, Stacy Medders and John R. Wright, Southeastern Okla. a State University, Durant, OK 74701.

The action of HNO_2 on 3-amino-L-tyrosine (3AT) and luminol in a mixed water/DMSO solvent leads to the diazonium derivatives of 3AT and luminol. If acetone precipitates of these diazotization products are allowed to stand for days at 25 C, a water soluble, anionic polymer is formed. The polymer shows promise in microwave and acoustic dosimetry applications. The ^1H -nmr spectrum of the polymer obtained from 3AT alone resembles that of 3AT (but broadened), and considerations of reactivity, the spectra and HMO calculations suggest a possible $\alpha\text{-C-NH-aryl}$ linkage. H,H-COSY spectra could reveal such a linkage, but all attempts to prepare low DP_n oligomers have failed.

(Supported by AFOSR Grant F49620-92-J-0191 DEF)

EFFECT OF H_2O_2 AND FORMATE ION ON THE SONOCHEMILUMINESCENCE OF A DIAZOTIZATION POLYMER OF LUMINOL AND 3-AMINO-L-TYROSINE, Staci Medders, Danny Rose, Phyllis Berryman and John R. Wright, Southeastern Oklahoma State University, Durant, OK 74701.

It has been known at length that alkaline solutions of luminol exhibit sonochemiluminescence when an acoustic field is applied, and the addition of certain anions such as carbonate enhance the effect. Other anions such as phosphate show similar enhancing effects. Small amounts of H_2O_2 also boost the sonic response. An anionic polymer prepared from diazotized mixtures of luminol and 3-amino-L-tyrosine (3AT) was found to quench sonochemiluminescence in all of the alkaline luminol/anion/ H_2O_2 systems tried except one. The exception was that of the formate ion, which with the polymer of luminol and 3AT and H_2O_2 produced a pronounced burst of light in the sonic field and could be the basis of an acoustive wave dosimeter. (Supported by AFOSR Grant F49620-92-J-0191 DEF)

SONOCHEMILUMINESCENT RESPONSE OF
ALKALINE LUMINOL SOLUTIONS CONTAINING
METAL OXIDE COLLOIDS, Danny Rose, Staci
Medders, Phyllis Berryman and John R.
Wright, Southeastern Oklahoma State
University, Durant, OK 74701.

Preliminary studies have shown that
certain finely-divided transition metal
oxide colloids cause modest enhancements
of the sonic chemiluminescent effect in
alkaline solutions of luminol. Of
fifteen different metals tested,
Nickel(II) produced the most marked
effect, a 29-fold increase (essentially
instantaneously) when 50 Watts of 20 kHz
sonic irradiation was applied to 50 mL
of the chemluminescent medium. The data
show fairly large fluctuations; in the
case of the nickel(II) system the
standard deviation was 13.9 for a mean
value of 29.1.

(Supported by AFOSR Grant F49620-92-J-
0191 DEF)

APPENDIX II - PUBLICATIONS

The appended reprint of a publication which appeared in a recent issue of Microchemical Journal originated in AFOSR funded research conducted prior the the current reporting period (though closely related to the ongoing objectives). The P.I. is currently working on a manuscript "Sonochemiluminescent Properties of a Polymer of Luminol and 3-Amino-L-tyrosine", which will also be submitted to Microchemical Journal. A structural characterization of the polymer, the main objective at this time, would of course lead to an additional paper.

Chemiluminescent Dosimetry of Microwave Heating and Acoustic Irradiations Based on Luminol and Metal Oxide Catalysts

TIM SMITH,* ANN MACKIE,* STEVEN VAN WAGGONER,* GREGORY GANDY,*
MELANIE WASHBURN,* ROBERT SELF,* ALICIA HORN,* JOHNATHAN L. KIEL,†
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Received March 6, 1992; accepted March 31, 1992

Solutions of luminol, hydrogen peroxide, and sodium hydroxide containing trace amounts of various metal oxide catalysts were examined for chemiluminescent sensitivity to both heating and acoustic irradiations. It was found that a system containing copper(II) produced the largest relative change in luminescence as a result of heating, where I/I_0 increased about 35-fold during a 40°C temperature increase beginning at 5°C. In contrast, a system catalyzed by scandium(III) was much less affected by heating but produced an immediate 2.5-fold luminescence increase in sonic irradiations at 5°C. These systems may have applications as chemiluminescent imaging dosimeters for distinguishing the heating and acoustic effects which occur when energetic pulses of microwave radiation are absorbed by material objects. Most of the metal-based dosimeters which were tried either reacted too rapidly or exhibited a weak temperature dependence, and a dosimeter based on luminol diazonium ion expended itself in seconds. © 1993 Academic Press, Inc.

INTRODUCTION

The goal of the work reported here has been to identify chemiluminescent dosimeters which exhibit increased light emission in response to temperature increases for the purpose of visualizing the spatial distribution of absorbed microwave energy as heat. These could be used to measure the effects of complex geometry and microwave wavelength on heating distributions in target phantoms. We have also evaluated the effects of acoustic fields on chemiluminescent solutions since part of the energy of an impinging microwave pulse is partitioned into an acoustic wave (1). Both heating and acoustic effects occur when water-containing targets absorb high powered microwave (HPM) energy, and the imaging dosimeters described here are intended for studies of HPM effects.

Aqueous solutions of the organic substance luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) undergo a well-known chemiluminescent reaction initiated by a variety of free radicals (2). Products of the reaction include 3-aminophthalic acid and molecular nitrogen along with a characteristic blue chemiluminescence. The reaction is a reasonable basis for imaging dosimetry, and we have tried a variety

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of initiating agents in the hope of finding dosimeters which would exhibit a steep temperature dependence of chemiluminescent intensity, or perhaps an enhanced sensitivity to an acoustic field. Several reacting systems involving transition metal oxide catalysts and H_2O_2 (which might be expected to produce free radicals) have been examined for their influence on this reaction and especially the temperature dependence of their capacity to initiate luminol's chemiluminescence. All of the chemiluminescent initiators reported here operate under alkaline conditions since luminol is insoluble in water at neutral pH.

MATERIALS AND METHODS

Measurements of Relative Chemiluminescent Intensity during Microwave-Induced Heating

It is recognized that measurements of specific absorption rates (SARs) are beset with many difficulties (3), e.g., dose distributions may not be uniform, convection may be a problem and temperature measurements are at best averages. In our beginning studies of these dosimeters an attempt was made to measure absolute values of chemiluminescent intensities, which led to much frustration with data reproducibility. The data reported here are relative intensities, I/I_0 , where I_0 is the observed intensity immediately before the beginning of a microwave irradiation and I is the intensity at any other time. This procedure led to much more consistent results.

Irradiations were carried out in a 2.4-GHz microwave oven (rated at approximately 500 W) which had been made light tight. Emphasis was placed on maintaining a fixed geometry and controlled initial conditions. The measuring cuvette contained 100 ml of the dosimeter solution, and this cell communicated to the luminometer through a nonmetallic fiber optic bundle. The oven was loaded with 800 ml of water divided into four 200-ml portions in a square pattern around the cell. These containers were also maintained in a fixed geometry, and each irradiation began with the four containers filled with water at an initial temperature of 23°C. By loading the oven in this fashion it was possible to maintain the dosimeter heating rate at 10°C/min. This heating rate was verified by digital thermometry using an Omega AET instrument, by inserting a low-mass thermocouple (type E probe) to the cuvette's midpoint after various irradiation times.

The choice of a 10°C/min heating rate approximates values that are easily achieved by both pulsed and continuous microwave sources and target volumes. For example, a pulsed source operating at an instantaneous power of 1 MW for 1×10^{-5} s yields 10 J/pulse, and each pulse will produce a 0.24°C temperature increment in a 10-ml water absorber, assuming complete conversion to heat. The chosen rate of 10°C/min is realized if the pulse recurrent rate is only 0.7/s, which is a very modest rate since even conventional microwave generators in multi-megawatt air traffic control radars operate at pulsewidths of a few microseconds and pulse recurrent rates of approximately 300 to 400/s. At the chosen 10°C/min heating rate the Cu(II) dosimeter described here was readily imaged using a charge-coupled device (CCD) camera, and the dosimeter's luminosity increased at about 1%/s (or 1% per 0.17°C). At the higher powers and pulse recurrent frequen-

cies it is possible to produce sudden and substantial increments of heating and corresponding increases in chemiluminescence. The uneven deposition of microwave energy in an irregularly shaped phantom may thus be recorded before convection and heat transfer have time to distort the chemiluminescent image.

Luminometric intensities were obtained by means of a Turner TD-20e luminometer interfaced to an IBM PS/2 (Model 30/286). An adapter head for the fiber optic bundle was fabricated locally.

Sonic Irradiations

Acoustic irradiations of aqueous suspensions were carried out using a Branson Model W-185 sonifier fitted with a compact conical tip. In order to avoid violent swirling, this transducer was operated at 50 W instead of its full power capacity. The irradiated volume was 100 ml, which resulted in a 7°C/min heating rate. This rate is comparable to the microwave studies described above; however, the observed sonochemiluminescent effects occur as virtually instantaneous changes when the acoustic field is switched on and are thus readily distinguished from the much slower increase of luminescence which occurs as the sample heats up. The measured values of I may be extrapolated back to the irradiation start time to obtain the instantaneous I/I_0 ratio, where I_0 is the intensity before starting the irradiation.

Dosimeter Preparations

Alkaline luminol was prepared fresh daily and consisted of 20.0 mg of luminol (Sigma) in 6.00 ml of 1.00 M NaOH, made with deionized water. A 3.00-ml aliquot of the alkaline luminol solution was made up to 50.0 ml in deionized water to obtain solution A. Transition metal salts were each prepared as a 2% solution by dissolving 1.00 g in 50 ml deionized water; in some cases these solutions had to be acidified to maintain solubility. Solution B was obtained by mixing a 250- μ l aliquot of the transition metal solution with 50 ml of 3% H₂O₂. Solutions A and B were brought to 5°C in a constant temperature bath and the mixed immediately before use. It should be noted that in most cases, owing to the dosimeter's alkalinity, the catalyst is a colloidal form of the hydrated oxide of the transition metal in question. If the metal ions were in a homogeneous solution, catalyst concentrations in these dosimeters would be on the order of 0.0001 M; however, for the reason just stated, it is not possible to specify a catalyst concentration. The other concentrations were as follows: hydrogen peroxide, 0.44 M; NaOH, 0.030 N; and luminol, 0.00057 M. In all experiments an arbitrary 2.0-min delay was imposed between the mixing of solutions A and B and the beginning of an irradiation.

RESULTS AND DISCUSSION

Theory

Most of the chemiluminescent dosimeters tested in this work were not responsive to heating or acoustic fields, but a few exhibited desirable characteristics. A desirable thermal dosimeter should produce the largest possible relative increase in luminosity, I/I_0 , in a given heating interval, where I_0 is the starting luminosity

and I the maximum luminosity attained after the interval. It was noted that of the dosimeters which responded to heating, those of a low initial luminosity exhibited an exponentially increasing luminosity/time relationship, while the initially more luminous dosimeters would rise to a peak of chemiluminescence and then fall back. If it is assumed that the rate of consumption of luminol is proportional to both the radical generation rate and the intensity of chemiluminescence, the behavior of the more luminous dosimeters is seen to be simply due to depletion of the reactant (luminol) during the heating interval. The following modeling of these systems is an attempt to identify the factors which maximize I/I_0 in a heat-sensitive dosimeter. The model is not applicable to the case of sonochemiluminescent dosimeters.

It is recognized that the various dosimeters considered here may act by different mechanisms to activate luminol's chemiluminescence, e.g., some may produce free radicals by decomposing H_2O_2 while others might involve direct interactions of luminol with the metal ion. In either case the chemiluminescence intensity is expected to be a function of both the concentration of luminol and the concentration of the initiator. Since the effective catalyst concentration is relatively low and presumed constant, its kinetic effects, especially the effects of temperature on the peroxide decomposition rate, are to be found in the apparent rate constant of luminol's chemiluminescent reaction.

A simple case for modeling this system would be a first-order process, i.e., $dC/dt = kC$, where C is the concentration of the chemiluminescent agent and k the apparent rate constant. The Cu(II) dosimeter, for example, exhibited a mixed first-order/zero-order decay of chemiluminescent intensity, I , for the first 40 min and then a purely zero-order process from 40 min onwards. This dosimeter chemiluminesces weakly for hours and is markedly temperature sensitive at all times. An Arrhenius expression may be substituted for k to account for the case of a system undergoing temperature changes:

$$dC/dt = A_0 \exp(-E_a/RT)C \quad (1)$$

Under the experimental conditions of this investigation, T may be approximated as $T = Bt + T_0$, where t is the elapsed time, B is the heating rate in K/min and the starting temperature, T_0 , is 278 K. The integration of Eq. (1) is not straightforward and involves an exponential integral and logarithmic integral treatment; however, numerical integrations using actual reaction conditions are simple (4), and a family of time versus luminosity curves is shown in Fig. 1 for an activation energy of 20 kcal/mol and four preexponential factors (similar families were constructed over a range of activation energies, but the activation energy in Fig. 1 approximates that of the Cu(II) dosimeter). The I/I_0 values of Fig. 1 are assumed identical to the ratio of reaction rates obtained from the integration of C as a function of time. For activation energies $E_a < 40$ kcal/mol, realistic figures for the preexponential factor A_0 are approximated by $10^{0.69 E_a}$.

One should note that in Fig. 1 the curves that turn up sharply without rolling over (low A_0 values) are, in practice, intrinsically the least luminous dosimeters, whereas those that peak and fall away are intrinsically bright. In the latter case the

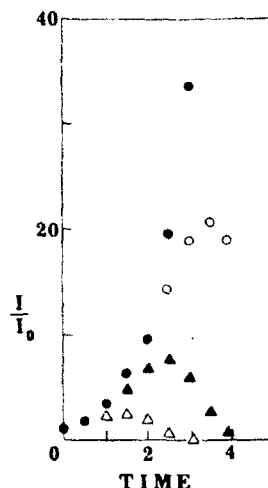


FIG. 1. Calculated values of relative chemiluminescent intensity (I/I_0) as a function of time for a heating rate of $10^\circ\text{C}/\text{min}$ and a first-order process. The time scale is in minutes, and heating begins at the origin. Predicted time curves are shown for an activation energy of 20 kcal/mol and several values of the preexponential factor, A_0 . Legend for A_0 : \bullet 1×10^{12} ; \circ 1×10^{14} ; \blacktriangle 3×10^{14} ; \triangle 1×10^{15} . Different values of A_0 do not affect the initial portion of the time curves, but downward deviations occur as the chemiluminescent material begins to be consumed. The lower curves represent larger A_0 values, and thus intrinsically more luminous dosimeters.

chemiluminescent agent is rapidly consumed in the reaction, and integrated light pulses of this type are the basis of most luminometric determinations (5). However, in the type of application envisioned here, the dosimeter should have a reasonably large activation energy (realistically, at least in the 12–15 kcal/mol range) and a preexponential factor that is neither too large nor too small, so that the dosimeter is consumed slowly at the beginning temperature but is luminous enough to image with a CCD camera. In other words, the dosimeter must emphasize the rising portion of the time curve, which is dominated by the Arrhenius expression rather than the concentration of the chemiluminescent species.

Numerical integrations were also tried for other reaction orders (zero-order, second-order, etc.), and the results were qualitatively similar to those of Fig. 1, with differences appearing mainly in the relative values of E_a and A_0 to obtain similar profiles of I/I_0 versus heating time. Thus, for luminometric applications of the type described here, the reaction order is not a primary consideration if the dosimeter is not significantly depleted during the measuring interval.

Measurements of Relative Luminosity versus Heating Time for Metal Catalyst Dosimeters

The H_2O_2 decomposition rate as evidenced by visible effervescence (bubbling) generally correlated with luminometer intensity; the ruthenium(III) and cobalt(II) dosimeters bubbled the most vigorously and were also the most luminous. Figure 2 presents time curves of I/I_0 for three of the dosimeters, and Table I collects the maximum I/I_0 values observed in a 4-min heating interval (a temperature increase

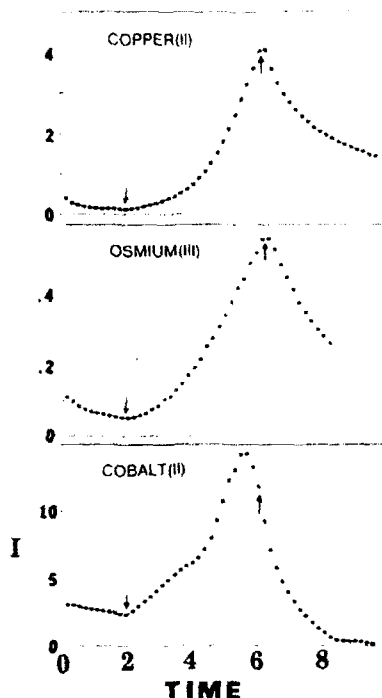


FIG. 2. Measured chemiluminescent intensity, I , as a function of time for the Cu(II), Os(III), and Co(II) dosimeters at a heating rate of $10^{\circ}\text{C}/\text{min}$ starting at 5°C . These are reproducible time curves selected from redundant data, and I is the arbitrary reading from the Turner luminometer, geometry being constant for all data. Microwave irradiation begins at 2 min and ends at 6 min in each recording (arrows). The copper(II) dosimeter produced the largest relative increase (i.e., I/I_0) and appears to be the best dosimeter of the 16 metal systems examined. The cobalt(II) dosimeter is sensitive to CO_2 and is the most luminous of the three shown here. The shoulder on the timecurve for cobalt(II) is not explained by the simple model.

of 40 K) for the various dosimeters examined in this work. The mean values and standard deviations of Table 1 are based on four replications; however, the more interesting dosimeters (e.g., those of Cu and Fe) have been replicated dozens of times. The water blank dosimeter (deionized water in place of a metal-containing solution) was more sensitive to heating than the Nb(IV), Ag(I), V(III), Hf(IV), Mo(II), and Y(III) systems, the latter five being essentially invariant with temperature changes. The best dosimeters are clearly those based on copper(II), osmium(III), iron(III), cobalt(II), and cobalt(III), and these are sufficiently luminous for imaging applications. Using the method of initial rates, i.e., by extrapolating chemiluminescence time curves back to the dosimeter starting times, the temperature dependence of the Cu(II) dosimeter's chemiluminescence gave a linear relationship for $\log I$ versus $1/T$ and indicated an activation energy of 19 kcal/mol. Comparing Table 1, Fig. 1, and Fig. 2 with this result, the data are consistent with a preexponential factor on the order of 10^{13} for a first-order process.

TABLE I
Thermal Sensitivities of Various Dosimeters

Source of metal oxide	III_0	SD
CuCl ₂	35.6	16.7
CoF ₃ ^a	12.2	2.9
OsCl ₃ (1:5 diluted)	8.6	1.3
FeSO ₄ ^b	7.4	1.1
Fe ₂ (SO ₄) ₃	7.6	1.0
CoSO ₄ ^a	6.2	1.3
NiCl ₂	4.4	0.9
ZrCl ₄	2.6	0.7
ScCl ₃	2.3	0.8
NbCl ₅	1	—
AgClO ₄	1	—
VCl ₃ ^a	1	—
HfCl ₄	1	—
MoBr ₂	1	—
YCl ₃	1	—
Water blank ^a	2.7	0.6

^a Sensitive to dissolved CO₂, which enhances luminescence.

^b Probably oxidizes to Fe(III) immediately.

Table 1 compares different oxidation states in some cases since these differences might have altered dosimeter characteristics. In practice, the catalyst's oxidation state is liable to change (e.g., the Fe(II) and Fe(III) systems).

The time curves for Cu(II) and Os(III) in Fig. 2 resemble those shown in Fig. 1 (upper curve), but a reproducible shoulder in the heating portion of the time curve suggests more complex processes for the Co(II) dosimeter. Note that in Fig. 2 the Os(III) dosimeter is a special case since the metal concentration is one-fifth that specified in the experimental part. A Ru(III)-based dosimeter (not in Table 1) was the most luminous but it expended itself quickly and produced a relatively modest variation of III_0 , thus resembling the lower curves of Fig. 1. The more luminous dosimeters have lower activation energies, and it was found that simple dilution of the catalyst, which decreases the apparent A_0 , did not result in large improvements of III_0 .

It should be noted that some of the dosimeters were sensitive to dissolved CO₂, an effect observed by others (6). The Co(II) dosimeter produces a striking burst of light when CO₂ is bubbled through it (quantitatively, approximately a threefold increase). This reaction might be used as the basis of a detector for dissolved CO₂; however, chemiluminescent dosimeters which are sensitive to dissolved gases (the amount of which might vary) are probably of questionable value in thermal imaging applications.

Luminol Diazonium Chloride

Some thought was given to the possibility of developing organic catalysts for dosimeter applications, and luminol may be activated by organic reagents. The

action of acidic sodium nitrite on luminol produces the diazonium salt, a dark red substance which may be partially purified and is stable enough to be isolated in the solid form. Aqueous solutions of this substance react with alkaline H_2O_2 (with no added metal ions), apparently producing a cascade of free radicals since a 50:50 mixture of luminol and its diazotized derivative produces a bright burst of blue chemiluminescence when the mixture is quickly combined with alkaline H_2O_2 . This reaction is interesting, but it is uncontrollably fast, apparently having a low activation energy, and is thus unsuitable for thermal dosimetry. It is possible that the reaction could be a less expensive alternative to metal ion initiation in luminol/ H_2O_2 chemical lasers (7).

Sonochemical Effects

The cell for microwave/thermal dosimetry was easily modified for acoustic irradiations. The cuvette and fiber optic assembly was relocated to a light-tight box, and the transducer of the sonifier was positioned 1.0 mm above and 1 mm laterally away from the fiber optic terminus (the positioning must be done accurately). The luminometer was set to sample at 0.2-min intervals, and the acoustic irradiation (50 W at 20 kHz) was started 2 min after mixing the dosimeter. The sonochemical luminescent effect was recorded as I/I_0 , as a function of time, where I_0 and I are, respectively, the measured chemiluminescent intensities immediately before and immediately after starting the irradiation. It is emphasized that this is an immediate effect and thus distinct from the progressive (acoustic) heating which follows. A typical plot of intensity versus time is shown in Fig. 3, and Table 2 compares five of the dosimeters (in three replications). The scandium dosimeter is seen to be the most sensitive to acoustic irradiations, whereas its response relative to the copper dosimeter was much less in the microwave (heating) irradiations (Table 1).

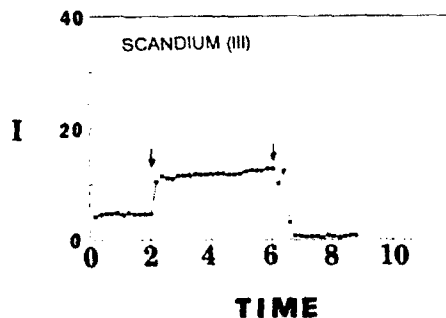


FIG. 3. Sonochemically induced chemiluminescence of the scandium(III) dosimeter, which is the one (of the 16 systems tried) which was found most sensitive to acoustic irradiations. Sonic irradiation begins at 2 min and ends at 6 min. The starting temperature is 5°C , and the luminescence increase during the irradiation period is presumed due to heating on the sample. The cause of the slow recovery after irradiation is not known, and these extended irradiations are not comparable to microsecond-range pulsed irradiations. A thoroughly dark-adapted eye can see the sonochemical luminescent glow of most of the dosimeters (near the transducer), and this glow appears and disappears instantaneously when the irradiation is briefly turned on and off.

TABLE 2
Measured Sonochemiluminescent Effects on Five
Dosimeters Which Responded to
Acoustic Energy

Source of metal	I/I_0	SD
ScCl ₃	2.5	0.2
CoSO ₄	2.2	0.2
ZrCl ₄	1.2	0.1
Fe ₂ (SO ₄) ₃	1.2	0.05
CuCl ₂	1.1	0.04

At first glance one might expect microwave and sonic irradiations to act similarly, i.e., by causing heating on the sample; however, the cavitation/compression effects of a strong acoustic field lead to large instantaneous deviations from the apparent sample temperature (8), whereas microwave energy is deposited more evenly in the sample through dielectric absorptions, where the quantum energy does not exceed kT . The two kinds of irradiations are thus not qualitatively alike, and some catalysts may be more effective in the production of radicals at the higher instantaneous temperatures of sonic irradiations. The evidence presented here appears to substantiate that expectation. Dosimeters with such differential characteristics might be of value in studies of the acoustic waves which are produced when high-powered microwave pulses encounter an absorbing object (1). It is noted that very energetic microwave pulses produce a temperature step, thus an increase in steady luminosity, whereas the acoustic effects associated with pulsed microwave absorption are inherently transient, lasting on the order of 10^{-5} to 10^{-4} s (depending on target size and sound velocity). Electronic circuits for separating faint flashes from a much brighter background have been described (9) and might be applicable in measurements of the transient acoustic effects reported here.

ACKNOWLEDGMENT

This work was funded by an Air Force Office of Scientific Research Grant (project S-210-9MG-037; award F49620-88-C-0053/SB5881-0378), which is gratefully acknowledged.

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